Safety Data Sheet

CCNU (Lomustine)

Division of Safety
National Institutes
of Health

WARNING!

THIS COMPOUND IS TOXIC, CARCINOGENIC, TERATOGENIC, MUTAGENIC, AND EMBRYOTOXIC. IT IS ABSORBED THROUGH THE RESPIRATORY AND INTESTINAL TRACTS AND TRANSPLACENTALLY. IT MAY IRRITATE THE SKIN AND EYES. AVOID FORMATION AND BREATHING OF AEROSOLS.

LABORATORY OPERATIONS SHOULD BE CONDUCTED IN A FUME HOOD, GLOVE BOX, OR VENTILATED CABINET.

AVOID SKIN CONTACT: IF EXPOSED, WASH WITH SOAP AND COLD WATER. AVOID WASHING WITH SOLVENTS. AVOID RUBBING OF SKIN OR INCREASING ITS TEMPERATURE.

FOR EYE EXPOSURE, IRRIGATE IMMEDIATELY WITH LARGE AMOUNTS OF WATER. FOR INGESTION, DRINK MILK OR WATER. REFER FOR GASTRIC LAVAGE. FOR INHALATION, REMOVE VICTIM PROMPTLY TO CLEAN AIR. REFER TO PHYSICIAN.

IN CASE OF LABORATORY SPILL, WEAR PROTECTIVE CLOTHING DURING CLEANUP. AVOID SKIN CONTACT OR BREATHING OF AEROSOLS. SEE CASTEGNARO ET AL. (1985) FOR DETAILS. DISPOSE OF WASTE SOLUTIONS AND MATERIALS APPROPRIATELY.

A. Background

CCNU (lomustine) is a yellow powder, stable in pure form and in solution at slightly acid pH, readily decomposed in alkaline solution. It is toxic in all mammalian species tested (oral and parenteral LD50 in the mg/kg range) and carcinogenic, mutagenic, teratogenic, and embryotoxic. Because of its high lipid solubility, which permits penetration of the "blood-brain barrier", its major use is as an antineoplastic, alone or in combination with other therapeutic agents, in the treatment of primary and metastatic brain tumors in addition to that of Hodgkin's

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disease, colorectal tumors and certain pulmonary malignancies. Toxic side effects are on the hematopoietic system, the gastrointestinal tract, liver, and kidney and the cardiopulmonary system in some species. Its mechanism of action consists of alkylating and carbamoylating reactions with nucleic acids, probably mainly after metabolic conversion to hydroxylated metabolites.

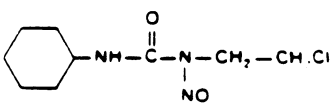
General reviews include: Carter et al. (1972), Schabel (1976), IARC (1981).

B. Chemical and Physical Data:

1. Chemical Abstract No.: 13010-47-4

2. Synonyms: CCNU; CeeNU; N-(2-chloroethyl)-N'-cyclohexyl-N-nitrosourea; lomustine; NCI-C04740; NSC-79037; urea, N-(2-chloroethyl)-N'-cyclohexyl-N-nitroso-.[A]

3. Chemical structure and molecular weight:



$C_9H_{16}ClN_3O_3$, 233.69

4. Density: No data.

5. Absorption spectroscopy: No data. Effluents of chromatographic columns are often monitored at 230 nm which is probably a measure of the alicyclic ring.

6. Volatility: No data; may be regarded as essentially non-volatile

7. Solubility: Very slightly soluble in water (less than 0.05 mg/ml) soluble in absolute ethanol (70 mg/ml), lipids and nonpolar organic solvents. Formulations for parenteral injections are usually in a 1:1 mixture of ethanol with Cremophor EL (Sigma) (a surfactant based on polyethoxylated ricinus oil) and then diluted with saline (e.g., Lee and Workman, 1983).

8. Description: Yellow powder.

9. Boiling point: No data; melting point: 90°C.

[A]Chemical Abstracts name, used for listing in 8th Decennial Index and subsequently.

0. Stability: Previous work on the stability of CCNU in aqueous solution has been reviewed recently (Bosanquet, 1985). Dry CCNU in unopened vials is stable at least two years at room temperature, (PDR, 1980) though storage at refrigerator temperature is recommended. It is also stable in non-polar organic solvents. There are very few data on stability of CCNU in aqueous solution; in the absence of such data, one may assume its stability to be similar to that of bis(2-chloroethyl) nitrosourea, i.e., maximal stability at pH 3.5-4 and marked decomposition at pH higher than 5. A scheme for its decomposition has been proposed (Colvin et al., 1976). See also Chemical reactivity, below.

1. Chemical reactivity: The decomposition of CCNU is markedly increased in the presence of proteins (Levin et al., 1978); the mechanism appears to be catalysis by serum albumin of the conversion of CCNU to reactive species. CCNU interacts with DNA by alkylation and carbamoylation (Lown et al., 1979), particularly with deoxyguanosine and deoxycytidine (Gombar et al., 1980), and carbamoylation of free amino groups of peptides and proteins (Wheeler et al., 1975). The cyclohexyl ring is subject to oxidation.

2. Flash point: No data.

3. Autoignition temperature: No data.

4. Explosive limits in air: No data.

Fire, Explosion, and Reactivity Hazard Data

1. CCNU is likely to be inactivated under conditions of fire. Fire-fighting personnel should wear protective clothing and face masks.

2. Flammability is likely to be low.

3. Conditions contributing to instability are acid, alkali, and elevated temperature.

4. Hazardous decomposition products under conditions of fire are likely to include hydrochloric acid and nitrogen oxides. The formation of 2-chloroethanol, acetaldehyde, vinyl chloride, ethylene, and cyclohexylamine in varying amounts has been reported for the aqueous hydrolysis of CCNU, and these compounds may also be decomposition products on ignition (Reed et al., 1975).

Operational Procedures

The NIH Guidelines for the Laboratory Use of Chemical Carcinogens describe operational practices to be followed when potentially carcinogenic chemicals are used in NIH laboratories. The NIH Guidelines should be consulted to identify the proper use conditions required and specific controls to be implemented during normal and complex operations or manipulations involving CCNU.

It should be emphasized that this data sheet and the NIH Guidelines are intended as starting points for the implementation of good laboratory practices when using this compound. The practices and procedures described in the following sections pertain to the Natio Institutes of Health and may not be universally applicable to other institutions. Administrators and/or researchers at other institutio should modify the following items as needed to reflect their indivi management system and current occupational and environmental regula

Solutions of CCNU penetrate various glove materials (Laidlaw et al. 1984). This factor should be taken into account when handling CCNU

1. Chemical inactivation: Validated methods have been reported (Castegnaro et al., 1985).

2. Decontamination: Turn off equipment that could be affected by CCNU or the materials used for cleanup. If there is any uncertainty regarding the procedures to be followed for decontamination, call the NIH Fire Department (dial 116) for assistance. Consult Castegnaro et al. (1985) for details concerning decontamination of surfaces, glassware, and animal cages.

3. Disposal: It may be possible to decontaminate waste streams containing CCNU before disposal. For details, see Castegnaro et al. (1985). No waste streams containing CCNU shall be disposed of in sinks or general refuse. Surplus CCNU or chemical waste streams contaminated with CCNU shall be handled as hazardous chemical waste and disposed of in accordance with the NIH chemical waste disposal system. Nonchemical waste (e.g., animal carcasses and bedding) containing shall be handled and packaged for incineration in accordance with the NIH medical-pathological waste disposal system. Potentially infectious waste (e.g., tissue cultures) containing CCNU shall be disinfected by heat using a standard autoclave treatment and packaged for incineration, as above. Burnable waste (e.g., absorbent bench top liners) minimally contaminated with CCNU shall be handled as potentially infectious waste and packaged for incineration, as above.

Absorbent materials (e.g., associated with spill cleanup) grossly contaminated shall be handled in accordance with the chemical waste disposal system. Radioactive waste containing CCNU shall be handled in accordance with the NIH radioactive waste disposal system.

4. Storage: Store solid CCNU in unopened vials, preferably under refrigeration. Avoid exposure to light and moisture. Store working quantities of CCNU and its solutions in an explosion-safe refrigerator in the work area. Sec B10 for further information.

E. Monitoring and Measurement Procedures including Direct Field Measurements and Sampling for Subsequent Laboratory Analysis

1. Sampling: It is important that blood samples be immediately cooled in ice, centrifuged while cold, and then extracted. Tissue samples are frozen and homogenized (Lee and Workman, 1983).

2. Analysis: Early analyses were based on colorimetry, employing the Griess or Bratton-Marshall reagent after acid liberation of nitrous acid in the presence of sulfanilamide (Loo and Dion, 1965; DeVita et al., 1967).[A] This method is capable of measuring plasma levels down to 1 µg/ml, a sensitivity which, because of the fast disappearance of intact CCNU from the blood stream, is not sufficient for monitoring patients for more than 5-10 minutes after oral or intravenous administration (Levin et al., 1978). In addition, the method also measures active metabolites of CCNU (Weinkam and Liu, 1982) unless further modified by extraction (Kari et al., 1980). Differential pulse polarography (Russo et al., 1984) has a detection limit of 20 ng and is more specific. Reversed phase high-pressure liquid chromatography with ultraviolet detection has been used for plasma analysis (Lee and Workman, 1983). Derivatization methods, either by reaction with methanol to form O-methyl-carbamate (Weinkam and Liu, 1982) or with trifluoroacetic anhydride (Smith et al., 1981), followed by gas chromatography - mass spectrometry, the latter with a sensitivity of 1-3 ng/ml plasma, have further enhanced specificity and sensitivity. A further modification of the latter method, using methane chemical ionization indicates even higher sensitivity (Smith and Cheung, 1982).

These methods were developed for bis(2-chloroethyl) nitrosourea but should be applicable to CCNU also.

Biological Effects (Animal and Human)

Absorption: CCNU is quickly absorbed and produces biological effects after parenteral (intravenous, intraperitoneal) injection and by ingestion. There are no data concerning percutaneous absorption of CCNU.

Distribution and pharmacokinetics: Because of the rapid chemical and biochemical decomposition and oxidative transformation of CCNU in plasma and tissues shortly after administration, distribution data, based on experiments with CCNU labeled with ^{14}C in either the ethylene, carbonyl, or cyclohexyl moieties refe to hydrolysis products rather than intact CCNU. After oral administration of variously labeled CCNU in man, radioactivity wa demonstrated within 10 minutes in plasma, with peak levels in 1-6 hours (Sponzo et al., 1973). Significant quantities of radio activity are found in all tissues including brain and cerebrospi fluid within a short time and are similar regardless of position of label or route of administration. Preferential distribution to fat, liver, and brain (Oliverio et al., 1970; Litterst et al. 1974; Levin et al., 1978; Russo et al., 1984). Pharmacokinetic data have been published (Lee et al., 1985).

Metabolism and excretion: CCNU rapidly disappears from plasma after oral or parenteral administration, and it has been assumed that its fate *in vivo* is similar to its decomposition in aqueous solution. Schemes for this decomposition, including suggested mechanisms for non-specific catalysis of decomposition by serum proteins (see also B11) have been published (Colvin et al., 1976; Wheeler et al., 1975; Weinkam et al., 1980) and these indicate the formation of chloroethyl carbonium ion as an alkylat agent, and cyclohexyl isocyanate as a carbamoylating agent. Excretion of radioactivity due to variously (ethylene, carbonyl, cyclohexyl) labeled CCNU is mainly in the urine; 30 and 60% of total administered CCNU radioactivity appears within 12 and 48 h respectively (Oliverio et al., 1970; Sponzo et al., 1973). There is also some biliary excretion and reabsorption from the gastrointestinal tract in some species, and some radio-activity appears as respiratory CO_2 from ethylene- and carbonyl- (but not from cyclohexyl-) labeled CCNU. Other urinary metabolites are cyclohexylamine and dicyclohexyl urea.

In addition to these essentially hydrolytic products of CCNU metabolism, there has also been demonstrated *in vitro* monohydroxylation of the cyclohexyl ring by rat liver microsomes to yield *cis*- and *trans*- hydroxylated derivatives in position 2, 3, and 4 of the cyclohexyl ring (Hilton and Walker, 1975). The same hydroxylation has been found after

parenteral administration of CCNU (reviewed by Kohlhepp et al., 1981); major urinary excretion products among these are the cis-4- and trans-4-hydroxylated products and these have also been demonstrated in plasma after ingestion of CCNU in man (Kari et al., 1980; Lee et al., 1985). The properties of some of these derivatives have been studied; they appear to be more toxic than CCNU but have better therapeutic indices. Their carbamoylating and alkylating properties differ from each other and from CCNU, depending on the position of the hydroxyl group (Johnston et al., 1975; Wheeler et al., 1977; Heal et al., 1978). Other urinary products of metabolism are thiol conjugates (thiodiacetic acid, S-carboxymethyl cysteine) derived from the alkylating species (Kohlhepp et al., 1981).

Toxic Effects: The acute LD50 of CCNU is between 38 and 72 mg/kg for mouse and rat via the oral, intravenous, intraperitonea and subcutaneous route when observed for 30 days. As with other alkylating agents, the onset of symptoms is prolonged and no deaths are observed earlier than 4-5 days after administration even of massive doses.

The toxic effects in dogs and monkeys have been described (Oliverio, 1973; Schaeppi et al., 1974; Perry and Yarboro, 1984) and consist of delayed bone marrow and lymphoid tissue toxicity, resulting in lymphopenia and neutropenia. Additionally, dogs develop pyrexia, septicemia, pneumonia, and occasionally transien hepatic toxicity (Henry et al., 1973). Effects on spermatogenesi in mice (Meistrich et al., 1982) have also been noted. Hemorrhag inflammation and skin ulceration occurs in guinea pigs after intr dermal injection, similar to what might occur in patients upon extravasation (Barr et al., 1981). Symptoms in man are mainly on the hematopoietic system with occasional nausea and vomiting. The mechanism of toxic (and anticarcinogenic) action consists of alkylation and carbamoylation of DNA (Connors and Hare, 1975; Lown et al., 1979) and proteins (Connors and Hare, 1974), resulting in decrease or lack of incorporation of precursors into DNA and RNA and inhibition of protein synthesis. Some of the hydroxylated derivatives of CCNU (see section 3, above) are more effective as antineoplastics than the parent compound and may represent the "active species" (Johnston et al., 1975).

Carcinogenic Effects: The literature through 1980 has been summarized (IARC, 1981). CCNU is carcinogenic in rats, producing lung carcinomas after intraperitoneal or intravenous injection (Zeller et al., 1982).

Mutagenic and Teratogenic Effects: CCNU is mutagenic in the Ames test, and some of its hydroxylated metabolic derivatives even

more so (Franza et al., 1980). It is also mutagenic against Chinese hamster cells (Bradley et al., 1980). It is teratoge in the rat and rabbit (Thompson et al., 1975).

Emergency Treatment

1. Skin and eye exposure: For skin exposure, remove contaminate clothing and wash skin with soap and water. Skin should not be rinsed with organic solvents. Avoid rubbing of skin or increasing its temperature. For eye exposure, irrigate immediately with copious quantities of running water for at least 15 minutes. Obtain ophthalmological evaluation.

2. Ingestion: Drink plenty of water or milk. Induce vomiting. Refer for gastric lavage.

3. Inhalation: Remove victim promptly to clean air. Administer rescue breathing if necessary.

4. Refer to physician.

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